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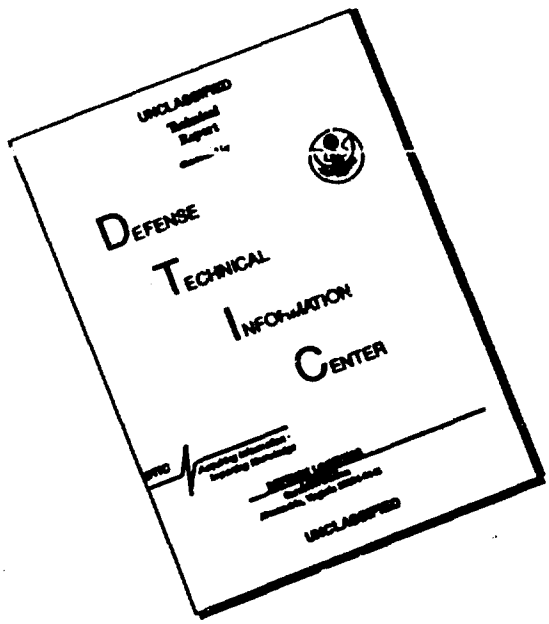

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MECHANISM OF DETOXIFICATION BY MEANS OF FORMOL

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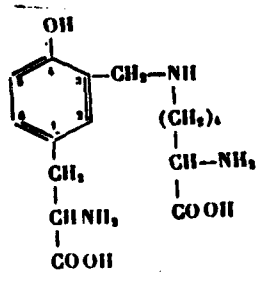
Mrs. Judith Blass, Mr. Bernard Bizzini and Mr. Marcel Raynaud, transmitted by Charles Cernez-Rieux

In the hydrolysis products of two pure anatoxins (diphtheric and tetanic) and of formolated derivatives of bovine serum-albumin, the authors established the presence of a special compound of the Mannich type, resulting from the combination of lysin and tyrosin through a methylenic group.

According to Fraenkel-Conrat and Olcott (1) the irreversible phase of the combination of formol with the proteins could result from various types of reactions which one of us discussed (2) and for which only some indirect proof has so far been supplied. Among the reactions produced, one involves a Mannich combination (3) between the ortho CH group of tyrosin and ε-NH_2 of lysin [Acid (amino-2-aminomethyl-6) 3'-[(hydroxy-4'-phenyl) alanin-1'] hexanoic]



We tried to furnish direct proof for the formation of these methylenic Mannich bridges:

(1) By preparing the model compound above (compound X);

(2) By trying to isolate pure diphtheric and tetanic anatoxins in the

hydrolysates which had been suitably prepared in advance (4).

1. The N-acetylated derivative of the model compound X was obtained by causing the formol to react with a equimolecular mixture of α-N-acetyl-lysin and of α-N-acetyl-tyrosin, for a period of 7 days at 37°C. The optimum conditions for the pH and the concentrations were determined. The reaction mixture was fractionated by two methods.

a. Chromatography on a roll of paper (in a chromax column, LKB) with development by means of a mixture of butanol and acetic acid. We found that we get a principal compound (corresponding to the derivative acetylated on the two amine functions of product X) and three or four minor derivatives corresponding to various secondary reactions. The main compound is completely deacetylated by the action of HCl, N/I for a period of 48 hours at 37°C. The methylenic combination [bond] created here is very resistant; it is not broken after the action of HCl, 5.7 N at 110° for a period of 48 hours nor after the action of soda 5 N for a period of 5 hours at 120°.

The principal deacetylated compound and the deactylated accessories derivations all have a more or less marked basic character and can be differentiated from each other by electrophoresis on paper at various pH. The disclosure was made by means of ninhydrin and the reagent of Folin (5).

b. The reaction mixture, from which the excess of free formol or formol reversibly bonded (6) or deactylated by the action of HCl, 5.7 N at 110° for a period of 24 hours, had been removed, was fractionated on a carbon column (7) and then on a column of resin Dowex 50x4 (8). The principal derivative is washed out by 0.5 M ammonium acetate with pH of 6.8 and it "leaves" the column in the position where free arginin passes.

2. We obtained a similar derivative by producing a reaction between formol and an equimolecular mixture of α-N-acetyl-tyrosin and of monochydrochloride of 14C-6-lysin (non-N-acetylated).

After separation according to the method described under 1, b, we obtained a principal radioactive derivative which established the presence of lysin in derivative X. The presence of the tyrosin nucleus is easy to establish through the spectral properties and the characteristic staining reactions: Folin reaction and Gerngross reaction (9).

3. We were able to identify the same derivative X in the products of the acid hydrolysis of the diphtheric and tetanic anatoxins and of the various types (2) of formolated bovine serum-albumin by their various properties (spectrum, behavior under electrophoresis on paper and under chromatography on paper and on Dowex 50x4).

This research remained negative in the case of the acid hydrolysates of the corresponding nonformolated proteins: pure diphtheric and tetanic toxins, bovine serum-albumin.

Conclusion. During the action of formol upon diphtheric and tetanic toxins, under empirically well-controlled conditions which lead to the formation of corresponding stable antatoxins, a certain fraction of the irreversibly bonded formol is fixed in the form of methylenic bridges, giving rise to a Mannich derivative containing lysin and tyrosin.

(This report was delivered to the 12 July 1965 session.)

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